SRI LANKA STANDARD 735: PART 7 /Section 4: 2017
(ISO 8968-4: 2016)
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METHODS OF TEST FOR
MILK AND MILK PRODUCTS
PART 7: DETERMINATION OF PROTEIN
SECTION 4: MILK - DETERMINATION OF PROTEIN AND NON-PROTEIN NITROGEN CONTENT AND TRUE PROTEIN CONTENT CALCULATION (REFERENCE METHOD)
(SECOND REVISION)

SRI LANKA STANDARDS INSTITUTION

Sri Lanka Standard

METHODS OF TEST FOR MILK AND MILK PRODUCTS

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Section 4: Milk - Determination of protein and non-protein nitrogen content and true protein content calculation (Reference Method)

(Second Revision)

SLS 735: Part 7: Section 4: 2017
(ISO 8968 - 4: 2016)

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Sri Lanka Standards are subject to periodical revision in order to accommodate the progress made by industry. Suggestions for improvement will be recorded and brought to the notice of the Committees to which the revisions are entrusted.

This Standard does not purport to include all the necessary provisions of a contract.

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NATIONAL FOREWORD

This Sri Lanka Standard was approved by the Sectoral Committee on Food Products and was authorized for adoption and publication as a Sri Lanka Standard by the Council of the Sri Lanka Standards Institution on 2017-07-21.

This Sri Lanka Standard was first published in 1989 as an adoption of ISO 8968-4 and subsequently revised in 2012. This Standard prescribes the general methods for determining whether the material conforms to the requirements of the relevant individual Standards and thus form a necessary adjunct to series of Sri Lanka Standard Specification for milk and milk products. However, keeping in view the experience gained during the years and various International Standards brought out by the International Organization for Standardization (ISO) on the subject of testing of milk and milk product, it was decided to revise it with a view to updating the existing methods of test.

In order to accommodate large number of test methods within the scope of the Standard, this Standard is published in several parts.

Part 7 of the standard consists of several sections.
Section 4 of this part of the Standard is identical with **ISO 8968- 4: 2016 (IDF 20-4:2016)** Milk – Determination of protein and non-protein nitrogen content and true protein calculation.(Reference method) published by the International Organization for Standardization (ISO) and International Dairy Federation (IDF).

TERMINOLOGY AND CONVENTIONS

The text of the International Standard has been accepted as a suitable for publication, without deviation, as a Sri Lanka Standard. However, certain terminology and conventions are not identical with those used in Sri Lanka Standard. Attention is therefore drawn to the following:

a) Wherever the words “International Standard” appear referring to this Standard, this should be interpreted as “Sri Lanka Standard”.

b) The comma has been used throughout as a decimal marker. In Sri Lanka Standards, it is the current practice to use the full point at the base line as the decimal marker.

c) Wherever page numbers are quoted, they are ISO page numbers.

Cross References

International Standard	Corresponding Sri Lanka Standard
ISO 8968-1 (IDF 20-1) Milk and milk products – Determination of nitrogen content – Part 1: Kjeldahl principle and crude protein calculation	SLS 735: Part 7: Section 1, Methods of test for milk and milk products – Determination of nitrogen content – Kjeldahl method

INTERNATIONAL STANDARD

ISO 8968-4

IDF 20-4

Second edition
2016-04-01

Milk and milk products — Determination of nitrogen content —

Part 4: Determination of protein and non-protein nitrogen content and true protein content calculation (Reference method)

Lait et produits laitiers — Détermination de la teneur en azote —

Partie 4: Détermination de la teneur en azote protéique et non protéique et calcul de la teneur en protéines vraies (Méthode de référence)

Reference numbers
ISO 8968-4:2016(E)
IDF 20-4:2016(E)

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Forewords

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products* and the International Dairy Federation (IDF). It is being published jointly by ISO and IDF.

This second edition of ISO 8968-4|IDF 20-4 cancels and replaces ISO 8968-4|IDF 20-4:2001 and ISO 8968-5|IDF 20-5:2001, which have been technically revised.

ISO 8968|IDF 20 consists of the following parts, under the general title *Milk and milk products — Determination of nitrogen content*:

— *Part 1: Kjeldahl principle and crude protein calculation*

— *Part 3: Block-digestion method (Semi-micro rapid routine method)*[1)]

— *Part 4: Determination of protein and non-protein nitrogen content and true protein content calculation (Reference method)*

1) It is intended that upon revision, the main element of the title of ISO 8968-3|IDF 20-3 (i.e. "Milk") will be aligned with the main element of the titles of ISO 8968-1|IDF 20-1 and ISO 8968-4|IDF 20-4.

IDF (the International Dairy Federation) is a non-profit private sector organization representing the interests of various stakeholders in dairying at the global level. IDF members are organized in National Committees, which are national associations composed of representatives of dairy-related national interest groups including dairy farmers, dairy processing industry, dairy suppliers, academics and governments/food control authorities.

ISO and IDF collaborate closely on all matters of standardization relating to methods of analysis and sampling for milk and milk products. Since 2001, ISO and IDF jointly publish their International Standards using the logos and reference numbers of both organizations.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. IDF shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute and endorsement

ISO 8968-4|IDF 20-4 was prepared by the IDF Standing Committee on *Analytical methods for composition* and ISO Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*.

The work was carried out by the IDF/ISO Project Group (C13) of the Standing Committee on *Analytical methods for composition* under the aegis of its project leaders, D. Barbano (US) and P. Trossat (FR).

This ISO|IDF International Standard cancels and replaces ISO 8968-4|IDF 20-4:2001 and ISO 8968-5|IDF 20-5:2001, which have been technically revised.

ISO 8968|IDF 20 consists of the following parts, under the general title *Milk and milk products — Determination of nitrogen content*:

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INTERNATIONAL STANDARD

ISO 8968-4:2016(E)
IDF 20-4:2016(E)

Milk and milk products — Determination of nitrogen content —

Part 4:
Determination of protein and non-protein nitrogen content and true protein content calculation (Reference method)

WARNING — The use of this part of ISO 8968|IDF 20 can involve the use of hazardous materials, operations and equipment. This part of ISO 8968|IDF 20 does not purport to address all the safety risks associated with its use. It is the responsibility of the user of this part of ISO 8968|IDF 20 to establish appropriate safety and healthy practices and determine the applicability of local regulatory limitations prior to use.

1 Scope

This part of ISO 8968|IDF 20 specifies a method for the direct and indirect determination of the protein nitrogen content of liquid, whole or skimmed milk.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 8968-1|IDF 20-1, *Milk and milk products — Determination of nitrogen content — Part 1: Kjeldahl principle and crude protein calculation*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
non-protein nitrogen content
NPN
mass fraction of substances determined by the specified procedure

Note 1 to entry: The non-protein nitrogen content is expressed as a percentage by mass.

3.2
protein nitrogen content
mass fraction of substances determined by the specified procedure, directly or, alternatively, indirectly

Note 1 to entry: The protein nitrogen content is expressed as a percentage by mass.

4 Principle

4.1 Indirect protein nitrogen

Precipitation of protein from a test portion by addition of trichloroacetic acid solution such that the final concentration of trichloroacetic acid in the mixture is approximately 12 %. Removal of the precipitated

milk protein by filtration, with the remaining filtrate containing the non-protein nitrogen components. Determination of the nitrogen content of the filtrate by the procedure described in ISO 8968-1|IDF 20-1.

Where the total nitrogen content of the milk sample has previously been determined, the true protein nitrogen content may be calculated as the difference between the total nitrogen content and the non-protein nitrogen content.

4.2 Direct protein nitrogen

Precipitation of protein from a test portion by addition of trichloroacetic acid solution such that the final concentration of trichloroacetic acid in the mixture is approximately 12 %. Separation of the protein precipitate by filtration. (The precipitate contains the protein nitrogen of the sample.) Determination of the nitrogen content of the precipitate by the procedure described in ISO 8968-1|IDF 20-1.

5 Reagents

5.1 General

Use only reagents of recognized analytical grade, unless otherwise specified, and distilled or demineralized water or water of equivalent purity.

The reagents specified for the determination of total nitrogen by the method described in ISO 8968-1 | IDF 20-1, together with the following, are required.

5.2 Trichloroacetic acid (CCl_3COOH) solution

Dissolve 15,0 g of trichloroacetic acid in water in a 100 ml one-mark volumetric flask. Dilute to the mark with water. Do not use concentrations of trichloroacetic acid and volumes of solutions other than those specified.

NOTE The performance of the method with respect to mean value and between-laboratory performance characteristics will be different, if using other than specified concentrations of trichloroacetic acid and volumes of solutions.

5.3 Hydrochloric acid standard volumetric solution

For the direct approach, the 0,1 mol/l hydrochloric acid solution is as described in ISO 8968-1| IDF 20-1.

For the indirect protein nitrogen approach, the following hydrochloric acid solution is required c(HCl) = (0,01 ± 0,000 1) mol/l in addition to the 0,1 mol/l hydrochloric acid solution required as described in ISO 8968-1|IDF 20-1.

6 Apparatus

Usual laboratory apparatus and that specified for the determination of total nitrogen described in ISO 8968-1|IDF 20-1 and, in particular, the following.

6.1 Water bath, capable of maintaining a temperature of between 38 °C and 40 °C.

6.2 Conical flasks, of capacity 125 ml (indirect approach only).

6.3 Pipettes, of capacities 5 ml, 10 ml and 20 ml.

6.4 Filter funnel, made of glass, of diameter 75 mm.

6.5 **Filter paper**, nitrogen free, of diameter 15 cm.[2)]

6.6 **Automatic pipette**, piston pump, capable of delivering 10 ml.

6.7 **Beakers**, of capacity 50 ml (indirect approach only).

7 Sampling

Sampling is not part of the method specified in this part of ISO 8968|IDF 20. A recommended sampling method is given in ISO 707|IDF 50.

A representative sample should have been sent to the laboratory. It should not have been damaged or changed during transport or storage.

8 Preparation of test sample

Warm the test sample to between 38 °C to 40 °C in the water bath (6.1). Cool the sample to room temperature while gently mixing the test sample immediately prior to weighing the test portion (9.1 or 10.2.1).

9 Procedure — Direct protein nitrogen approach

9.1 Test portion

Pipette 5,0 ml ± 0,1 ml of the prepared test sample (see Clause 8) either into a dry and clean Kjeldahl flask or digestion tube, pre-weighed to the nearest 0,1 mg. Weigh the test sample to the nearest 0,1 mg. Immediately add 5,0 ml ± 0,1 ml of water to the flask or tube, rinsing any test sample on its neck into its bottom.

NOTE The use of either a Kjeldahl flask or a digestion tube is dependent on the laboratory's choice of digestion apparatus.

9.2 Determination

9.2.1 Precipitation and filtration

Add 40 ml ± 0,5 ml of trichloroacetic acid solution (5.2) to the Kjeldahl flask or digestion tube containing the test portion (9.1) and swirl to mix the contents. Let the flask or tube stand for approximately 5 min to allow the precipitate to settle. Pour the contents of the flask or tube through a filter paper (6.5) placed in a filter funnel (6.4). Collect the filtrate in a clean conical flask. Some of the precipitate will remain in the Kjeldahl flask or digestion tube and some will be collected on the filter paper. It is not necessary to remove all of the precipitate from the flask or tube.

Immediately after pouring the mixture and so as not to allow any precipitate to dry on the neck of the flask or tube, add by means of an automatic pipette (6.6), 10 ml of the trichloroacetic acid solution (5.2). Use the solution to rinse any precipitate from the neck of the flask or tube down into the bottom. Swirl to mix the contents. Pour the thus-obtained contents of the flask or tube through the same filter paper. Add the filtrate to that previously collected in the conical flask. Again, immediately rinse the neck of the flask or tube with a further 10 ml of trichloroacetic acid solution and swirl to mix the contents. Pour the contents of the flask or tube for the third time through the same filter paper, adding the filtrate to that collected previously in the conical flask.

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2) Whatman No 1 is an example of a suitable product available commercially. This information is given for the convenience of users of this part of ISO 8968|IDF 20 and does not constitute and endorsement by ISO or IDF of the product named. Equivalent products may be used it they can be shown to lead to the same results.

The obtained filtrate shall be clear and free of particulate matter. At this point, the filtrate is no longer needed and may be discarded in an appropriate manner.

If duplicated tests of the same test sample are to be carried out, two separate precipitation and filtration procedures shall be carried out for each test sample.

9.2.2 Preparation of the precipitate

Wearing gloves, carefully remove the filter paper from the filter funnel and fold the paper to enclose the precipitate. If any precipitate remains on either the inner or outer lip of the Kjeldahl flask or digestion tube, wipe with the folded filter paper so that any precipitate adheres to the paper and then drop the filter paper into the Kjeldahl flask or digestion tube.

9.2.3 Digestion and distillation

Add the appropriate amount of boiling aids (optional), potassium sulfate, copper catalyst solution and sulfuric acid to the Kjeldahl flask or digestion tube and continue with the digestion and distillation procedure specified in ISO 8968-1|IDF 20-1, either 9.1 or 9.2 depending on the type of Kjeldahl apparatus being used.

9.2.4 Titration

Titrate the liberated ammonia by using the procedure specified in ISO 8968-1|IDF 20-1.

9.3 Blank test

Carry out a blank test, taking a filter paper (6.5) washed with trichloroacetic acid solution (5.2) instead of the test portion as described in 9.2.1 and proceed to 9.2.3. Always titrate the blanks with the same reagent and apparatus as used for the test portions.

Keep a record of blank values. If blank values change, identify the cause.

9.4 Calculation and expression of results

9.4.1 Calculation of protein nitrogen content

Calculate the protein nitrogen content, w_{pn}, using Formula (1):

$$w_{pn} = \frac{1,400\,7 \times (Vs - Vb) \times M_r}{m} \quad (1)$$

where

w_{pn} is the protein nitrogen content of the sample, expressed as a percentage (mass fraction);

V_s is the numerical value of the volume of the hydrochloric acid standard volumetric solution used in the determination of ISO 8968-1|IDF 20-1, in millilitres, expressed to at least the nearest 0,05 ml;

V_b is the numerical value of the volume of the hydrochloric acid standard volumetric solution used in the blank test of ISO 8968-1|IDF 20-1, in millilitres, expressed to at least the nearest 0,05 ml;

M_r is the numerical value of the exact molarity of the hydrochloric acid standard volumetric solution (5.3), expressed to four decimal places. If sulfuric acid is substituted for hydrochloric acid, M_r is the exact molarity of the sulfuric acid multiplied by a factor of 2, expressed to four decimal places;

m is the numerical value of the mass of the test portion (9.1), in grams, expressed to the nearest 0,1 mg.

9.4.2 Expression of results

Express the results obtained to four decimal places, if needed for further calculations. In case they are end results, express those obtained for the nitrogen content to three decimal places, and for the protein content to two decimal places.

The results should not be rounded further until the final use of the test value is made. This is particularly true when the values are going to be used for additional calculation. One example is when the individual test values obtained from the analysis of many sample materials are used to calculate method performance statistics for within- and between-laboratory variation. Another example is when the values are used as a reference for instrument calibration (e.g. infrared milk analyser) where the values from many samples will be used in a simple or multiple regression calculation. In such cases, the results should not be rounded before they are used for further calculations.

9.4.3 Calculation of true protein content

Calculate the true protein content, w_{tp}, using Formula (2):

$$w_{tp} = w_{pn} \times 6{,}38 \quad (2)$$

where

w_{tp} is the true protein content, expressed as a percentage (mass fraction);

w_{pn} is the protein nitrogen content of the sample, expressed as a percentage (mass fraction) to four decimal places (9.4.1);

6,38 is the generally accepted multiplication factor to express the protein nitrogen content as true protein content.

9.4.4 Expression of the true protein content results

Express the results obtained for the true protein content to three decimal places, if needed for further calculations. In case these are the end results (see 9.4.2), express those results to two decimal places.

10 Procedure — Indirect protein nitrogen approach

10.1 Nitrogen determination

Determine the total nitrogen content of the test sample (see Clause 8), w_n, expressed as a percentage (mass fraction), using the procedure as described in ISO 8968-1|IDF 20-1.

10.2 Non-protein nitrogen determination

10.2.1 Test portion

Transfer 10,0 ml ± 0,1 ml of the prepared sample (see Clause 8) into a pre-weighed conical flask (6.3) and re-weigh to the nearest 0,1 mg.

10.2.2 Precipitation and filtration

Add 40 ml ± 0,5 ml (or equivalent mass) of trichloroacetic acid solution (5.2) to the conical flask (6.2) containing the test portion (10.2.1). Weigh the flask and its contents to the nearest 0,1 mg. Swirl to mix. Let the flask stand for approximately 5 min to allow the precipitate to settle.

Filter the contents of the conical flask through a filter paper (6.5) placed in a filter funnel (6.4). Collect the entire filtrate in a clean, dry conical flask (6.2). The filtrate shall be clear and free of particulate matter. If it is not, repeat the process of precipitation and filtration with a new test portion.

If duplicated tests of the same test sample are to be carried out, two separate precipitation and filtration procedures have to be carried out for each test sample.

10.2.3 Preparation of the filtrate

Swirl the filtrate in the conical flask (6.2) to ensure that it is mixed. Pipette 20 ml of the filtrate into a 50 ml beaker (6.7) and weigh. Pour the filtrate from the beaker into a Kjeldahl flask or digestion tube containing boiling aids (optional), potassium sulfate and copper sulfate (II) solution specified in ISO 8968-1|IDF 20-1. Immediately reweigh, to the nearest 0,1 mg, the empty beaker.

10.2.4 Digestion and distillation

Add the appropriate amount of sulfuric acid to the Kjeldahl flask or digestion tube and continue with the digestion and distillation procedure specified in ISO 8968-1|IDF 20-1, either 9.1 or 9.2 depending on the type of Kjeldahl apparatus being used.

10.2.5 Titration

Titrate the liberated ammonia by using the procedure specified in ISO 8968-1|IDF 20-1, but replacing the 0,1 mol/l hydrochloric acid standard volumetric solution by the 0,01 mol/l hydrochloric acid standard volumetric solution (5.3) as specified in Clause 5.

10.3 Blank test

Digest, distill and titrate blanks comprising of 0,1 g of sucrose and 16 ml ± 0,5 ml of trichloroacetic acid solution (5.2). Carry out a blank test following the procedure described in ISO 8968-1|IDF 20-1. Always titrate blanks with the same reagent and apparatus as used for the test portions.

Keep a record of blank values. If blank values change, identify the cause.

10.4 Calculation and expression of results

10.4.1 Calculation of non-protein nitrogen content

Calculate the non-protein nitrogen content, w_{npn}, by using Formula (3):

$$w_{npn} = \frac{1,4007 \times (V_s - V_b) \times M_r}{m_f \times m_m / (m_t - 0,065 \times m_m)} \quad (3)$$

where

w_{npn} is the non-protein nitrogen (NPN) content of the sample, expressed as a percentage (mass fraction);

V_s is the numerical value of the volume of the hydrochloric acid standard volumetric solution (5.3) used in the determination of ISO 8968-1|IDF 20-1, in millilitres, expressed to at least the nearest 0,05 ml;

V_b is the numerical value of the volume of the hydrochloric acid standard volumetric solution (5.3) used in the blank test of ISO 8968-1|IDF 20-1, in millilitres, expressed to at least the nearest 0,05 ml;

M_r is the numerical value of the exact molarity of the hydrochloric acid standard volumetric solution (5.3), expressed to four decimal places. If sulfuric acid is substituted for hydrochloric acid, M_r is the exact molarity of the sulfuric acid multiplied by a factor of 2, expressed to four decimal places;

m_f is the numerical value of the mass of 20 ml of filtrate (10.2.3), in grams to the nearest 0,1 mg;

m_m is the numerical value of the mass of the test portion (10.2.1), in grams, expressed to the nearest 0,1 mg;

m_t is the numerical value of the mass of the test portion after the addition of 40 ml of trichloroacetic acid solution (10.2.2), in grams expressed to the nearest 0,1 mg;

0,065 is the multiplication factor, based on the assumption that milk contains a mass fraction of about 3,5 % fat and 3,0 % true protein (thus, 0,035 + 0,030 = 0,065).

NOTE The multiplication factor 0,065 in the denominator might need to be adjusted if liquid dairy products of different composition are analysed (concentrated or fractionated skim or whole milk products, etc.).

10.4.2 Calculation of protein nitrogen content

Calculate the protein nitrogen content, w_{pn}, using Formula (4):

$$w_{pn} = w_n - w_{npn} \quad (4)$$

where

w_{pn} is the protein nitrogen content of the test sample, expressed as a percentage (mass fraction);

w_n is the nitrogen content of the test sample, expressed as a percentage (mass fraction);

w_{npn} is the non-protein nitrogen (NPN) content of the test sample, expressed as a percentage (mass fraction).

10.4.3 Expression of results

Express the results obtained to four decimal places, if needed for further calculations. In case these are the end results, express those results obtained for the non-protein nitrogen content to three decimal places, and for the non-protein content to two decimal places.

The results should not be rounded further until the final use of the test value is made. This is particularly true when the values are going to be used for additional calculation. One example is when the individual test values obtained from the analysis of many sample materials are used to calculate method performance statistics for within and between laboratory variation. Another example is when the values are used as a reference for instrument calibration (e.g. infrared milk analyser) where the values from many samples will be used in a simple or multiple regression calculation. In such cases, the results should not be rounded before they are used for further calculations.

10.4.4 Calculation of true protein content

Calculate the true protein content, w_{tp}, using Formula (2) in 9.4.3.

10.4.5 Expression of the true protein content results

Express the true protein content results as described in 9.4.4.

11 Precision

11.1 Interlaboratory test

The values for the repeatability and reproducibility were derived from the result of an interlaboratory test carried out according to ISO 5725-1 and ISO 5725-2. Details of the interlaboratory test of the method are summarized in References [4] and [5] and in the tables in Annex A. The values derived from this test might not be applicable to concentration ranges and matrices other than those given.

11.2 Repeatability

Non-protein nitrogen: the absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in not more than 5 % of cases be greater than:

— a mass fraction of nitrogen of: 0,002 5 %, or

— a mass fraction of non-protein of: 0,016 %.

True protein: the absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in not more than 5 % of cases be greater than:

— a mass fraction of protein nitrogen of: 0,006 3 %, or

— a mass fraction of true protein of: 0,040 %.

11.3 Reproducibility

Non-protein nitrogen: the absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will in not more than 5 % of cases be greater than the following:

— a mass fraction of nitrogen of: 0,005 2 %, or

— a mass fraction of non-protein of: 0,033 %.

True protein; the absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will in not more than 5 % of cases be greater than the following:

— a mass fraction of protein nitrogen of: 0,013 %, or

— a mass fraction of true protein of: 0,083 %.

12 Test report

The test report shall specify:

a) the method used, with reference to this part of ISO 8968|IDF 20, i.e. ISO 8968-4|IDF 20-4;

b) all the information required for the complete identification of the sample;

c) the sampling method used, if known;

d) all operating details not specified in this part of ISO 8968|IDF 20, or regarded as optional, together with details of any incident which might have influenced the result(s);

e) the test result(s) obtained;

f) if the repeatability has been checked, the final quoted result obtained;

g) if the recovery has been checked, the final quoted result obtained.

Annex A
(informative)

Results of a collaborative study — Estimation of precision data of direct and indirect methods for the true protein determination

A collaborative study was carried out with using nine fresh raw milk samples from individual farms, analysed in duplicate by 10 laboratories by both the indirect and the direct method as reported in Reference [5]. The published data analysis showed only a small bias between the two methods. It was decided that a global analysis of all the data could be performed with the results of both methods together to provide common precision data. The statistical analysis was carried out according to ISO 5725-2. See Table A.1.

The outlier detection pointed out six outliers by Cochran and one outlier by Grubbs resulting in six data pairs being discarded. There was no observed relationship between precision data and the true protein concentration level allowing then to calculate overall repeatability and reproducibility.

Table A.1 — Repeatability and reproducibility data from the collaborative study[5] of the method

Sample	1	2	3	4	5	6	7	8	9	Overall
No. of analysis	20	18	19	19	18	19	20	20	19	172
Mean Y (in g/100 g)	2,993	2,966	2,894	2,967	2,920	3,287	2,741	2,976	3,113	2,984
s_L **(in g/100 g)**	0,026	0,026	0,029	0,020	0,024	0,031	0,021	0,026	0,028	0,026
s_r **(in g/100 g)**	0,010	0,011	0,018	0,020	0,013	0,007	0,015	0,015	0,015	0,014
s_R **(in g/100 g)**	0,028	0,028	0,034	0,028	0,027	0,032	0,026	0,030	0,032	0,030
r (in g/100 g)	0,028	0,030	0,051	0,055	0,037	0,020	0,041	0,041	0,041	0,040
R (in g/100 g)	0,078	0,079	0,095	0,079	0,077	0,089	0,072	0,083	0,089	0,083
RSD_r **(in %)**	0,3	0,4	0,6	0,7	0,4	0,2	0,5	0,5	0,5	0,5
RSD_R **(in %)**	0,9	1,0	1,2	0,9	0,9	1,0	0,9	1,0	1,0	1,0
r rel (in %)	0,9	1,0	1,8	1,9	1,3	0,6	1,5	1,4	1,3	1,3
R rel (in %)	2,6	2,7	3,3	2,7	2,6	2,7	2,6	2,8	2,9	2,8

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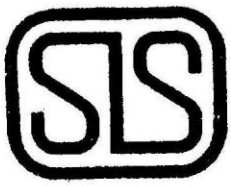

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